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**Effect of n-3 fatty acids on patients with advanced lung cancer: a double-blind, placebo-controlled study.**

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1 **Effect of n-3 fatty acids on patients with advanced lung cancer: a double-blind placebo-**  
2 **controlled study**

3

4 Concetta Finocchiaro\*, Olivia Segre\*, Maurizio Fadda\*, Taira Monge\*, Mara Scigliano\*, Marina  
5 Schena\*\*, Marco Tinivella\*\*\*, Elisa Tiozzo\*\*\*, Maria G. Catalano°, Mariateresa Pugliese°,  
6 Nicoletta Fortunati\$, Manuela Aragno°, Giuliana Muzio°, Marina Maggiora°, Manuela Oraldi°,  
7 Rosa A. Canuto°°

8

9 \*Department of Clinical Nutrition – San Giovanni Battista Hospital – Turin, Italy

10 \*\* Department of Oncology - San Giovanni Battista Hospital – Turin, Italy

11 \*\*\*Division of Clinical Nutrition – San Luigi Hospital – Orbassano (Turin), Italy

12 ° Department of Clinical Pathophysiology, University of Turin, Italy.

13 \$ Oncological Endocrinology, San Giovanni Battista Hospital – Turin, Italy

14 °°Department of Experimental Medicine and Oncology, University of Turin, Italy.

15

16 Address for Corresponding Author:

17 Dr.ssa C. Finocchiaro

18 Servizio di Dietetica e Nutrizione Clinica

19 Az. Ospedaliera-Universitaria San Giovanni Battista Torino

20 C.so Bramante 88/90, 10126 Torino

21 e-mail: [cfinocchiaro@molinette.piemonte.it](mailto:cfinocchiaro@molinette.piemonte.it)

22 Telephone number: 0039 0116336491, Fax number: 0039 011679477

23

24 Key words: lung tumour, cachexia, n-3 fatty acids, inflammatory parameters

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28

29 **Background:** PUFAs from fish oil appear to have anti-inflammatory and anti-oxidative effects and  
30 improve nutritional status in cancer patients.

31 **Objective:** the aim of the study was to investigate the effect of eicosapentaenoic acid (EPA) plus  
32 docosahexaenoic acid (DHA), on inflammatory condition, oxidative and nutritional status in  
33 patients with lung cancer.

34 **Design:** in our multicentre, randomised, double blind trial, 33 patients with a diagnosis of advanced  
35 inoperable non small cell lung cancer and undergoing chemotherapy were divided into two groups,  
36 receiving 4 capsules/day containing 510 mg of EPA and 340 mg of DHA, or 850 mg of placebo, for  
37 66 days. At the start of chemotherapy (T<sub>0</sub>), after 8 days (T<sub>1</sub>), 22 days (T<sub>2</sub>), and 66 days (T<sub>3</sub>),  
38 biochemical (inflammatory and oxidative status parameters) and anthropometric parameters were  
39 measured in both groups.

40 **Results:** a significant increase of body weight in the n-3 group at T<sub>3</sub> versus T<sub>0</sub> was observed.  
41 Concerning inflammation, C-reactive protein and IL-6 levels differed significantly between the n-3  
42 and placebo groups at T<sub>3</sub>, and progressively decreased during chemotherapy in the n-3 group,  
43 evidencing n-3 PUFAs' anti-inflammatory action. Concerning oxidative status, plasma reactive  
44 oxygen species levels increased in the placebo group versus the n-3 group at the later treatment  
45 times. Hydroxynonenal levels increased in the placebo group during the study, while they stabilized  
46 in the n-3 group.

47 **Conclusions:** our data confirm that the continual assumption of EPA plus DHA determined an anti-  
48 inflammatory and anti-oxidative action which could be considered a preliminary goal in anti-  
49 cachectic therapy.

50

## 51 *Introduction*

52

53 The role of fish-oil supplementation in numerous diseases has been emphasised; these include  
54 coronary disease, rheumatoid arthritis, inflammatory diseases, and cancer (1, 2, 3, 4, 5, 6).

55 Fish-oil supplementation has also been proposed for the treatment of cancer cachexia syndrome, an  
56 altered metabolic state characterized by anorexia, weight loss, asthenia, anaemia and alterations in  
57 carbohydrate, lipid and protein metabolism (7, 8). This syndrome is the major cause of morbidity  
58 and mortality in patients with advanced cancer (9).

59 Previous studies using conventional nutrition have shown that it is impossible to increase the lean  
60 tissue in cachexia patients (10); therefore it is important to use natural substances possessing both  
61 nutritional and anti-cachectic properties (11). In particular, eicosapentaenoic acid (EPA), an n-3  
62 PUFA present in large amounts in fish oil, can be considered as a potential natural support: it has  
63 been shown to have anti-inflammatory properties, down-regulating both pro-inflammatory cytokine  
64 production and the acute-phase protein response in cancer patients (12, 13, 14). Pro-inflammatory  
65 cytokines, IL-1, IL-6, and TNF- $\alpha$ , are recognized to play a central role in the pathogenesis of  
66 cancer-related cachexia (15, 16). Furthermore, EPA has also been shown to inhibit activation of the  
67 ubiquitin proteasome pathway, by the proteolysis-including factor, a cachectic factor produced by  
68 cancer tissue, which induces atrophy of skeletal muscle in animal models (9).

69 In 2006 (17) the administration of a dose of 2 g or 4 g of EPA was compared to placebo in 518  
70 cancer patients (gastrointestinal and lung), over an 8-weeks period. The results indicated that there  
71 was no benefit with the 4 g dose, but a potentially clinically relevant treatment effect with 2 g EPA  
72 per day.

73 In recent years, many studies have addressed this subject, but without reaching any conclusions  
74 concerning survival improvement and weight increase. This failure might be attributed to sample  
75 heterogeneity, reduction of patient number at the end of studies, lack of patient compliance, or  
76 subclinical toxicity of the higher dose of EPA (18).

77 The Cochrane analysis published in 2009 (19) concluded that there was insufficient evidence to  
78 draw any conclusions about EPA supplementation in cancer patients with cachexia. This systematic  
79 review also suggested there is little evidence of harm from using EPA, especially when combined  
80 with Megestrol Acetate.

81 The anti-inflammatory properties of EPA might also be involved in reducing oxidative stress. The  
82 association between reactive oxygen species (ROS), carcinogenesis, and progression of lung cancer  
83 has been widely demonstrated. The high percentage of oxidants in cigarettes smoke contributes to  
84 smoking-associated carcinogenesis (20). Asbestos fibres alter DNA of lung cells, with increased  
85 cell proliferation (21), by changing the redox state of the cells. Several mechanisms are responsible  
86 for the development of oxidative stress in cancer patients: the altered energy metabolism caused by  
87 the impossibility of normal nutrition in patients with anorexia, nausea and vomiting results in a  
88 reduced availability of glucose, proteins and vitamins, leading to increased free radicals (22, 23,  
89 24). Furthermore, chronic non-specific activation of the immune system, with excessive production  
90 of inflammatory cytokines, is responsible for increased production of ROS (25). An additional  
91 mechanism leading to oxidative stress derives from the use of anti-neoplastic therapy: many  
92 chemotherapies, and in particular alkylating agents and cisplatin, determine an increasing of ROS  
93 (26).

94 The aim of the present study was to investigate the effect of fish-oil components, namely EPA plus  
95 docosahexaenoic acid (DHA), versus placebo, on inflammatory condition, oxidative and nutritional  
96 status, in patients with lung cancer

97

98

99 *Experimental Methods and Participants*

100

101 **Study design**

102 The study was a multicentre, randomised, double-blind trial conducted between May 2007 and May  
103 2008. This study was conducted according to the guidelines laid down in the Declaration of  
104 Helsinki and all procedures involving patients were approved by the Scientific Ethics Committee of  
105 the City of Turin, Italy (research protocol n° Eudra-CT 2006-002978-21). Written informed consent  
106 was obtained from all patients. Patients were randomised at enrolment using a sequential series of  
107 numbered sealed envelopes containing computer-generated random assignments. A copy of the  
108 randomisation sequence was kept in a locked cabinet apart from the study personnel. Study  
109 products were packaged identically and were not distinguishable from one another.

110 Participants were randomly divided into two groups: the placebo group and the n-3 group (Figure  
111 1). The first group was provided with a daily dose of four capsules containing 850 mg of placebo  
112 (olive oil), the second with a daily dose of four capsules containing 510 mg of EPA and 340 mg of  
113 DHA, for 66 days (the entire period of chemotherapy).

114 Olive oil was selected as placebo because of evidence that ingestion of this oil was unlikely to  
115 change the fatty acid composition of either plasma or cellular phospholipids. Self-reported capsule  
116 intake was used to determine patient compliance.

117 Participants visited the research unit four times: at baseline ( $T_0$ ), after 8 days ( $T_1$ ), 22 days ( $T_2$ ), and  
118 66 days ( $T_3$ ), at which times a blood sample was taken and measurements were made (Figure 1).  $T_0$   
119 coincided with the start of chemotherapy.

120

## 121 **Eligibility criteria**

122 Baseline characteristics of patients recruited into this study are shown in Table 1.

123 Patients were recruited with a clinical diagnosis of advanced inoperable non small cell lung cancer,  
124 in the 18 to 70 year age range, and with 10% or less weight loss over the last three months, before  
125 the start the study. Patients received 3 courses of chemotherapy with Cisplatin and Gemcitabine to  
126 the following schedule: baseline ( $T_0$ ), after 8 days ( $T_1$ ), 22 days ( $T_2$ ), and after 66 days ( $T_3$ ). Life  
127 expectancy was two months or longer; Karnofsky Performance Status was 80 or higher.

128

129 **Exclusion criteria**

130 Patients were excluded if they had undergone chemotherapy failure, if metastases were present, if  
131 they were diabetic, had cardiovascular or infectious diseases, previous cancer (less than 5 years  
132 before or with relapse signs) or inflammatory disease. Patients with neurological deficiency or  
133 psychiatric diseases were also excluded.

134

135 **Nutritional status and dietary intake**

136 Patients were weighed on spring balance scales (Tanita Solar Powered Scale) without shoes and  
137 wearing light clothing. BMI was calculated as the ratio of body weight to the square of their height  
138 ( $\text{kg/m}^2$ ) (27).

139 In our study we didn't measure lean body mass with bioelectrical impedance analyser.

140 At the start of the study, pre-illness weight, unintentional weight loss over the last 6 months, and  
141 height were recorded.

142 Both group received the same dietary counselling with the aim to increase their energy and protein  
143 intake. A three day dietary recall was performed prior to assessment at baseline (week 0), and each  
144 week in the period of 66 days to assess the patients' dietary intakes. Patients completed a dietary  
145 diary, and were instructed by a dietician how fill them out correctly. Data on food intake reported  
146 by dietary diaries were then translated into energy and protein intakes by means of specific tables  
147 validated for Italian foods (28). Patients were also requested to record the number of capsules of **n-3**  
148 **fatty acids or olive oil** supplement taken each day.

149

150 **Blood analysis**

151 Plasma and erythrocytes were obtained by centrifuging venous blood (collected in tubes containing  
152 EDTA and kept on ice until separation) at 3000xg for 5 min at 4°C (centrifuge J6M, Beckman, Palo  
153 Alto, CA, USA), and stored at -80°C until use. The percentage content of different fatty acids was



154 determined in the total lipids extracted from plasma, and in phospholipids (PLs) extracted from  
155 erythrocytes membranes (29).

156 Albumin was analyzed by an autoanalyzer, whereas prealbumin (TBPA) and transferrin were  
157 detected quantitatively by immunoturbidity assay. C-reactive protein (CPR), IL-6, TNF $\alpha$  and PGE2  
158 production were evaluated in plasma by the ELISA method (R&D systems, Minneapolis, MN,  
159 USA). The levels of reactive oxygen species (ROS) were detected in the plasma using the probe  
160 2',7'-dichlorofluorescein diacetate (DCFH-DA) and measured fluorimetrically (30).  
161 Hydroxynonenal (HNE) concentration was determined on plasma by the method of EsterBauer et  
162 al. (31).

163

#### 164 **Statistical Analysis**

165 Results are expressed as means  $\pm$  S.D. Significant differences between patients in the n-3 and  
166 placebo groups were assessed by the unpaired T test, whereas significant differences within groups  
167 were assessed by the paired T test, all tests being two-sided. Statistical analyses were performed  
168 using the statistical software package SPSS for Windows Version 17.0 (SPSS, Chicago, Illinois,  
169 USA) at baseline (T<sub>0</sub>), after 8 days (T<sub>1</sub>), 22 days (T<sub>2</sub>), and 66 days (T<sub>3</sub>).

170

171

#### 172 *Results*

173

174 The series comprised 33 participants between 46 and 70 years old; they were randomly assigned to  
175 the placebo or the n-3 group. Baseline characteristics (T<sub>0</sub>) of patients are shown in Table 1.  
176 Fourteen participants, aged 50-70 years (mean age 60.57  $\pm$  7.43 years), were allocated to the  
177 placebo group; 19 participants, aged 46-69 years (mean age 58.10  $\pm$  6.72 years), were allocated to  
178 the n-3 group; 6 patients in the n-3 group (6 of 19: 31%) dropped out during the double-blind phase  
179 (2 patients changed department oncology, 2 refused treatment, 2 had diarrhea with capsules) Thus

180 13 participants in the n-3 group, (age 46-66 years; mean  $55.56 \pm 7.35$  years) completed the study  
181 (Figure 1).

182

### 183 **Nutritional Status**

184 The patients' nutritional status was not severely compromised, partly because some patients were  
185 overweight at the start of chemotherapy. Mean weight of the n-3 group was 75.10 kg ( $\pm 16.12$ ) at  
186 baseline and 78.50 kg ( $\pm 15.94$ ) at T<sub>3</sub>: an increase of 3.4 kg occurred, which was statistically  
187 significant (Figure 2). In the placebo group there was no increase: mean weight was 68.00 kg ( $\pm$   
188 12.85) at baseline and 68.92 ( $\pm 13.44$ ) at T<sub>3</sub> (Figure 2). **There** was no statistical significance  
189 between the two groups at T<sub>3</sub>.

190 Both groups had a satisfying calorie intake (**1.02 g/kg in n-3 group and 0.93 g/kg** in placebo group)  
191 but they took a different amount of proteins daily. Data for BMI and dietary intake (calories and  
192 proteins) revealed no statistically significant differences between the two groups. There was a non-  
193 significant increase in daily calorie and protein intake in the n-3 group (Table 1).

194 Nutritional blood parameters such as albumin, TBPA and transferrin did not differ between the two  
195 groups (data not shown).

196

### 197 **Percentage content of n-3 fatty acids in plasma and erythrocytes**

198 The percentage content of EPA in total lipids from plasma and in PLs from erythrocyte membranes  
199 is reported in Figure 3, which shows that EPA increased significantly in both plasma (panel A) and  
200 erythrocyte membranes (panel B) for the n-3 group, compared to the placebo group, at the two later  
201 experimental times.

202 No significant difference was evident in the percentage content of EPA in the placebo group at any  
203 of the experimental times, for either plasma or erythrocytes, whereas in the n-3 group there was a  
204 significant increase in EPA between the T<sub>1</sub> and T<sub>0</sub>, T<sub>2</sub> and T<sub>0</sub>, T<sub>3</sub> and T<sub>0</sub>, confirming the  
205 consumption of supplementary capsules.

206 The percentage content of DHA, in total lipids from plasma and in PLs from erythrocyte  
207 membranes, is reported in Figure 3 (panels C and D), which shows that DHA increased  
208 significantly in the plasma (panel C), but not in the erythrocyte membranes (panel D) for the n-3  
209 group, compared to the placebo group. Considering the percentage content of DHA in the placebo  
210 group throughout the experimental time, no variation was evident for either plasma or erythrocytes,  
211 whereas in the n-3 group, significant variations were detected, only for the plasma, between the T<sub>1</sub>  
212 and T<sub>0</sub>, T<sub>2</sub> and T<sub>0</sub>, T<sub>3</sub> and T<sub>0</sub>.

213 The percentage content of docosapentaenoic acid (DPA), which is an intermediate between the EPA  
214 and DHA, was also measured, showing no significant change in the plasma and in the erythrocyte  
215 membranes from placebo and n-3 groups at all experimental times.

216

#### 217 **Inflammatory parameters**

218 Figure 4 shows the trends of CPR, IL-6, TNF- $\alpha$ , and PGE<sub>2</sub>. In the n-3 group, CPR was not  
219 significantly changed between T<sub>0</sub> and T<sub>3</sub> (from 12.89 mg/l to 10.09 mg/l), while in the placebo  
220 group the increase, from 11.50 mg/l at T<sub>0</sub> to 27.09 mg/l at T<sub>3</sub>, was significant. Comparing the two  
221 groups at T<sub>3</sub>, the difference was statistically significant (Figure 4, panel A).

222 IL-6 values decreased at T<sub>3</sub> from their T<sub>0</sub> values in the n-3 group, and increased in the placebo  
223 group. Comparing the two groups at T<sub>3</sub>, the difference was statistically significant (Figure 4, panel  
224 B). TNF- $\alpha$  were higher in the placebo group than in the n-3 group, although not significantly so,  
225 possibly due to subject variability (Figure 4, panel C). Since n-3 PUFAs are able to inhibit the  
226 production of pro-inflammatory PGE<sub>2</sub>, this prostaglandin was evaluated in the plasma, using the  
227 ELISA test. A significant decrease in the n-3 group occurred during treatment, but no variation in  
228 the placebo group (Figure 4, panel D).

229

#### 230 **Oxidative status**

231 Since n-3 PUFAs could be damaged by ROS with the production of HNE, both HNE (panel A) and  
232 ROS (panel B) were evaluated in the plasma (Figure 5). Both these parameters increased at the later  
233 experimental times in the placebo group, whereas they decreased in the n-3 group. The HNE  
234 decrease in the n-3 group was statistically significant between T<sub>0</sub> and T<sub>3</sub>, and the difference between  
235 the two groups was statistically significant at T<sub>3</sub>. ROS values were significantly less in n-3 group  
236 than in the placebo group, at the latter two experimental times.

237

238

239 *Discussion*

240

241 Several studies (32, 33, 34) have emphasized that conventional nutritional support can only partially  
242 stop lean mass reduction in cancer patients: it is only possible to increase muscular mass by  
243 resolving metabolic alterations (35). Nutritional support is often wasted due to the hyper-metabolic  
244 state of the inflammatory pattern, and for this reason, in cancer patients, the first target of nutritional  
245 therapy should be to reduce the inflammatory state; n-3 PUFAs could have this effect (36, 37). For  
246 this reason, our study looked at the effect of n-3 PUFA administration in patients with advanced  
247 lung cancer, in order to evidence the ability of these compounds to improve patients' nutritional  
248 status and reduce the inflammatory and oxidative pattern. In this trial, EPA and DHA, two PUFAs  
249 present in fish oil, were administered in combination, rather than EPA alone, as in most studies.  
250 Administration was in capsules rather than supplements, as occurred in Fearons' trials (9, 17), and  
251 adherence to the study protocol was observed more closely. Detailed, consistent and persistent  
252 dietetic counselling, with assessment of any disorders connected with capsule assumption  
253 throughout the study, helped to obtain good compliance with the therapy and with the nutritional  
254 intake. Patients' observance of dietetic recommendations was confirmed by the increase, in the  
255 plasma of the n-3 group, of EPA and DHA percentage contents from T<sub>0</sub> to T<sub>3</sub>, compared with the  
256 placebo group. Changes in DHA content were not due to variation on DPA percentage content,

257 since the percentage content of this fatty acid did not show significant differences in all  
258 experimental times and in both groups.

259 The patients did not take other dietetic supplements unless n-3 fatty acids or olive oil capsules.

260 In regard to the nutritional status, we evidenced a slight increase, although not significant, of daily  
261 calorie and protein intakes during the study in the n-3 group, from start to end of chemotherapy,  
262 while calorie and protein intakes stabilized in the placebo group.

263 The difference between two groups are random, in fact already the n-3 fatty acid group at T0 took  
264 more calories and this trend continued throughout the time. Moreover, a statistically significant  
265 increase in body weight was achieved in the n-3 group at T<sub>3</sub> versus T<sub>0</sub>. It is not feasible that the  
266 slight increase of daily calorie and protein intakes may influence the variation in body weight.

267 These data are particularly interesting compared to reports in the literature, which are not always  
268 univocal on this point: some studies (9, 10, 38, 39) that have examined the effect of fish oil in  
269 cachectic patients expressed the opinion that valid conclusions are difficult to draw, for several  
270 reasons (short duration of trial, poor tolerability of supplementation, inability of patients to  
271 complete the study). Also the recent Cochrane (19) review about EPA for treatment of cancer  
272 cachexia did not confirm or reject the use of EPA in clinical practice; the results of the systematic  
273 review suggest that there is little evidence of harm deriving from the use of EPA. On the contrary,  
274 other studies have reported that EPA + DHA, as in our research, or EPA alone, reduce weight loss  
275 in patients with advanced cancer (33, 40, 41). In the case of other studies (9, 17, 40-42) a  
276 comparison with this research is difficult, because of methodological differences (lung, pancreatic  
277 or gastrointestinal cancer, treated with pure EPA).

278 A reduction of inflammatory parameter values found in the n-3 group versus the placebo group,  
279 although not always statistically significant, was observed. For example, CPR and IL-6 levels  
280 showed a significant difference between n-3 and placebo groups at T<sub>3</sub> ( $p < 0.05$ ) and a progressive  
281 decrease during chemotherapy in the n-3 group, evidencing an anti-inflammatory action of n-3  
282 PUFAs. On the contrary, variations in TNF $\alpha$  were not significant, and those of PGE<sub>2</sub> (expression of

283 pro-inflammatory factors) were statistically significant ( $p<0.05$ ) from  $T_0$  to  $T_2$  and  $T_3$  in the n-3  
284 group, but not between the two groups.

285 Some studies (10, 33, 43) have reported that n-3 PUFAs may suppress inflammatory cytokines in  
286 patients with advanced cancers; our data confirm this result . Van der Meij et al (43) recently  
287 published the results of a randomised, case-control, double-blind trial of 40 patients with stage III  
288 NSCLC, who received chemotherapy and radiotherapy, together with either supplements containing  
289 2 gr of EPA or isocaloric control supplements. After five weeks of treatment they observed that  
290 levels of inflammatory markers had decreased during chemotherapy and that IL-6 production was  
291 lower in the intervention group than in the control group.

292 To evaluate the oxidative status throughout the period of the trial reported here, ROS and HNE  
293 levels were determined. Plasma ROS levels were higher in the placebo group than in the n-3 group  
294 at the later treatment times ( $p<0.05$ ). HNE levels (expression of the injury from cellular oxidation)  
295 significantly ( $p<0.05$ ) increased in the placebo group during the study, while they stabilized in the  
296 n-3 group; this demonstrates the cellular oxidative effect of chemotherapy drugs, and the probable  
297 protective action of EPA + DHA. The difference between the two groups at the end of the study  
298 was also statistical significant.

299 Our data are encouraging with regard to the goals achieved, although the number of patients was  
300 limited: a statistically significant increase in body weight together with a reduction of inflammatory  
301 and oxidative parameters in the n-3 group confirm that the continual assumption of EPA + DHA  
302 showed an anti-inflammatory and anti-oxidative action, which might be considered a preliminary  
303 goal in anti-cachectic therapy.

304

305

306 *Conclusions*

307

308 Although numerous studies have addressed this subject and there is great interest in scientific  
309 research concerning n-3 fatty acids, there is as yet little clinic proof to justify applying the results to  
310 cancer patients.

311 In our randomised, double-blind study, despite the small number of patients, we analysed clinical,  
312 inflammatory and oxidative status during a period of 66 days, until the end of chemotherapy.

313 From this we may conclude that:

- 314 - fewer patients dropped-out than did from other studies;
- 315 - compliance with the dieticians' recommendations, and with the EPA and DHA assumption,  
316 were good;
- 317 - body weight increased significantly in the n-3 group;
- 318 - a significant reduction in inflammatory indexes and in oxidative status was observed.

319

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321

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323 All authors have read and agreed to the editorial policies, and declare that there are no financial  
324 conflicts of interest that might be construed to influence the results or interpretation of their  
325 manuscript.

326

327

#### 328 *Contribution of:*

329

330 C. Finocchiaro designed research,

331 T. Monge, M. Scigliano, M. Tinivella and E. Tiozzo conducted research,

332 M. Aragno, , M. Maggiora, M. Oraldi and M. Schena provided essential reagents and patients,

333 M. Pugliese performed TNF $\alpha$ ; M.G. Catalano and N. Fortunati analysed data.

334 M. Fadda and G. Muzio analysed data and performed statistical analysis,  
335 O. Segre wrote paper,  
336 M. Canuto and C. Finocchiaro had primary responsibility for final content.  
337  
338 All author read and approval the final manuscript. None of the authors had conflicts of interest.  
339  
340



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442 43. Van der Meij B, Langius J, Smit E *et al* (2010) Oral Nutritional Supplements Containing (n-  
443 3) Polyunsaturated Fatty Acids Affect the Nutritional Status of Patients with Stage III Non-  
444 Small Cell Lung Cancer during Multimodality Treatment. *J Nutr* **140**, 1774-1780.  
445

446 TABLE 1 - Nutritional status and calorie and protein intakes at baseline and at study end (T<sub>0</sub> and  
 447 T<sub>3</sub>)

		n-3 group (n= 13)		Placebo group (n= 14)	
		(M/F 8/5)	SD	(M/F 11/3)	SD
<b>Weight loss %</b>	T <sub>0</sub>	3.0	3.5	2.0	3.3
	T <sub>3</sub>				
<b>Karnofsky</b>					
<b>Performance Status</b>	T <sub>0</sub>	80	10	80	10
	T <sub>3</sub>				
<b>BMI (kg/m2)</b>	T <sub>0</sub>	26.19	6.98	25.25	3.92
	T <sub>3</sub>	27.65	7.02	25.58	4.43
<b>Calorie intake/day</b>	T <sub>0</sub>	8072.47	1596.3	7371.46	1421.95
	T <sub>3</sub>	1990.56	393,44	1685.57	169.32
<b>(Kjoule/Kg)(Kcal/d</b>	T <sub>0</sub>				
	T <sub>3</sub>	8840.08	1147.7	7131.32	1016.10
<b>ay)</b>	T <sub>0</sub>	2160	330.8	1730.29	242.69
	T <sub>3</sub>				
<b>Protein intake/day</b>	T <sub>0</sub>	68.56	15.03	60.00	8.5
	T <sub>3</sub>	74.88	17.33	61.43	8.52
<b>(g/day)</b>	T <sub>0</sub>	119.31	26.85	110.63	23.64
	T <sub>3</sub>	29.22	6.38	26.42	5.65
<b>Calorie intake/Kg</b>	T <sub>0</sub>	120.48	26.69	109.32	17.08
	T <sub>3</sub>	29.63	7	26.11	4.08
<b>(Kjoule /Kg)</b>	T <sub>0</sub>	1.02	0.29	0.94	0.24
	T <sub>3</sub>	1.04	0.32	0.94	0.16
<b>(K/Kg)</b>	T <sub>0</sub>				
	T <sub>3</sub>				
<b>Protein intake/Kg</b>	T <sub>0</sub>				
	T <sub>3</sub>				
<b>(g/kg)</b>	T <sub>0</sub>				
	T <sub>3</sub>				

448

449 The weight loss percentage is related to the last three months, before the start of the study.

450 **Figure legends**

451

452 FIGURE 1 – Study design

453

454 FIGURE 2 – Comparison of weight changes (Kg) in the placebo and n-3 groups during the  
455 experimental time

456 Data are expressed as means  $\pm$  S.D. \* “paired t test”  $p < 0.05$   $T_3$  versus  $T_0$ .

457 (–▲–) placebo group, (–□–) n-3 group

458

459 FIGURE 3 – Comparison of changes in EPA and DHA in plasma and in erythrocyte membrane  
460 content in the placebo and n-3 groups during the experimental time

461 Data are expressed as means  $\pm$  S.D. and are the percentage content of EPA in plasma (panel A) and  
462 erythrocyte membranes (panel B), and the percentage content of DHA in plasma (panel C) and  
463 erythrocyte membranes (panel D).

464 \* “paired t test”  $p < 0.05$   $T_1$ ,  $T_2$ ,  $T_3$  versus  $T_0$ .

465 § “unpaired t test”  $p < 0.05$  n-3 group versus placebo group.

466 (–▲–) placebo group, (–□–) n-3 group

467

468 FIGURE 4 – Comparison of changes in CPR, IL-6, PGE2 and TNF- $\alpha$  content in plasma in the  
469 placebo and n-3 groups during the experimental time

470 Data are expressed as means  $\pm$  S.D. and are the plasma content of CPR (panel A), IL-6 (panel B),  
471 and PGE2 (panel C).

472 \* “paired t test”  $p < 0.05$   $T_2$ ,  $T_3$  versus  $T_0$ .

473 § “unpaired t test”  $p < 0.05$  n-3 group versus placebo group.

474 (–▲–) placebo group, (–□–) n-3 group

475

476 FIGURE 5 – Comparison of changes in HNE and ROS content in plasma in the placebo and n-3  
477 groups during the experimental time  
478 Data are expressed as means  $\pm$  S.D. and are the plasma content of HNE (panel A) and ROS (panel  
479 B).  
480 \* “paired t test”  $p < 0.05$   $T_3$  versus  $T_0$ .  
481 § “unpaired t test”  $p < 0.05$  n-3 group versus placebo group.  
482 (—▲—) placebo group, (—□—) n-3 group



Figure 1

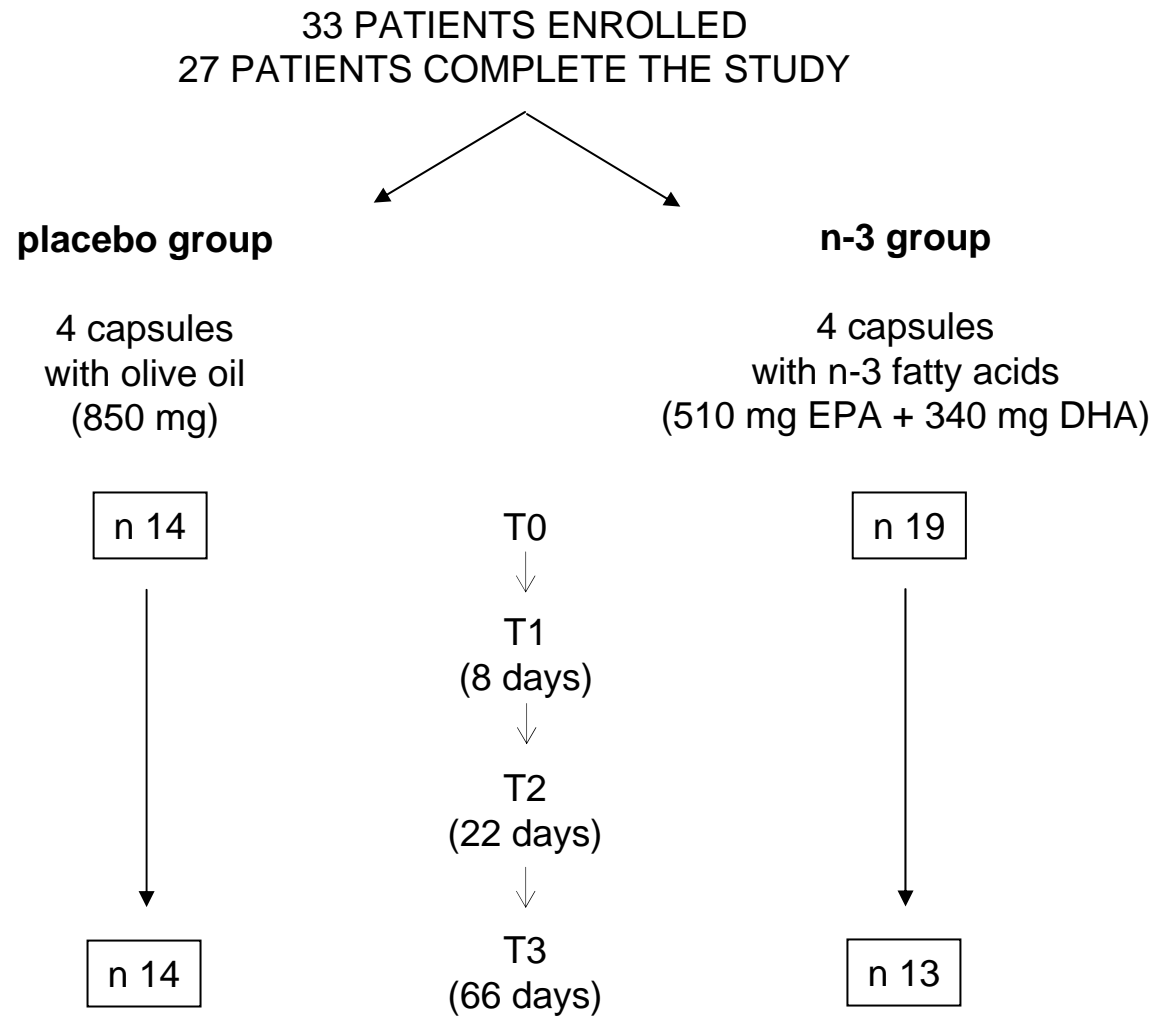


Figure 2

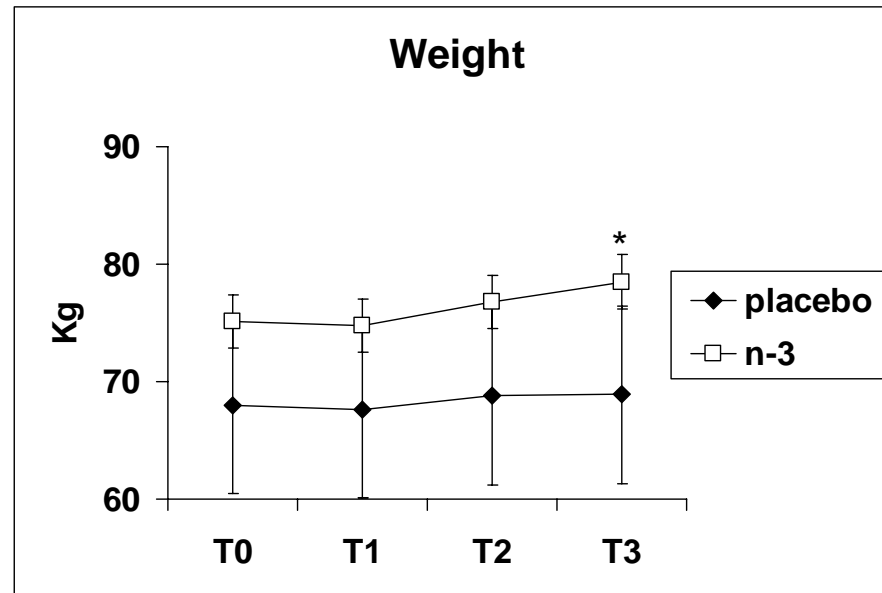


Figure 3

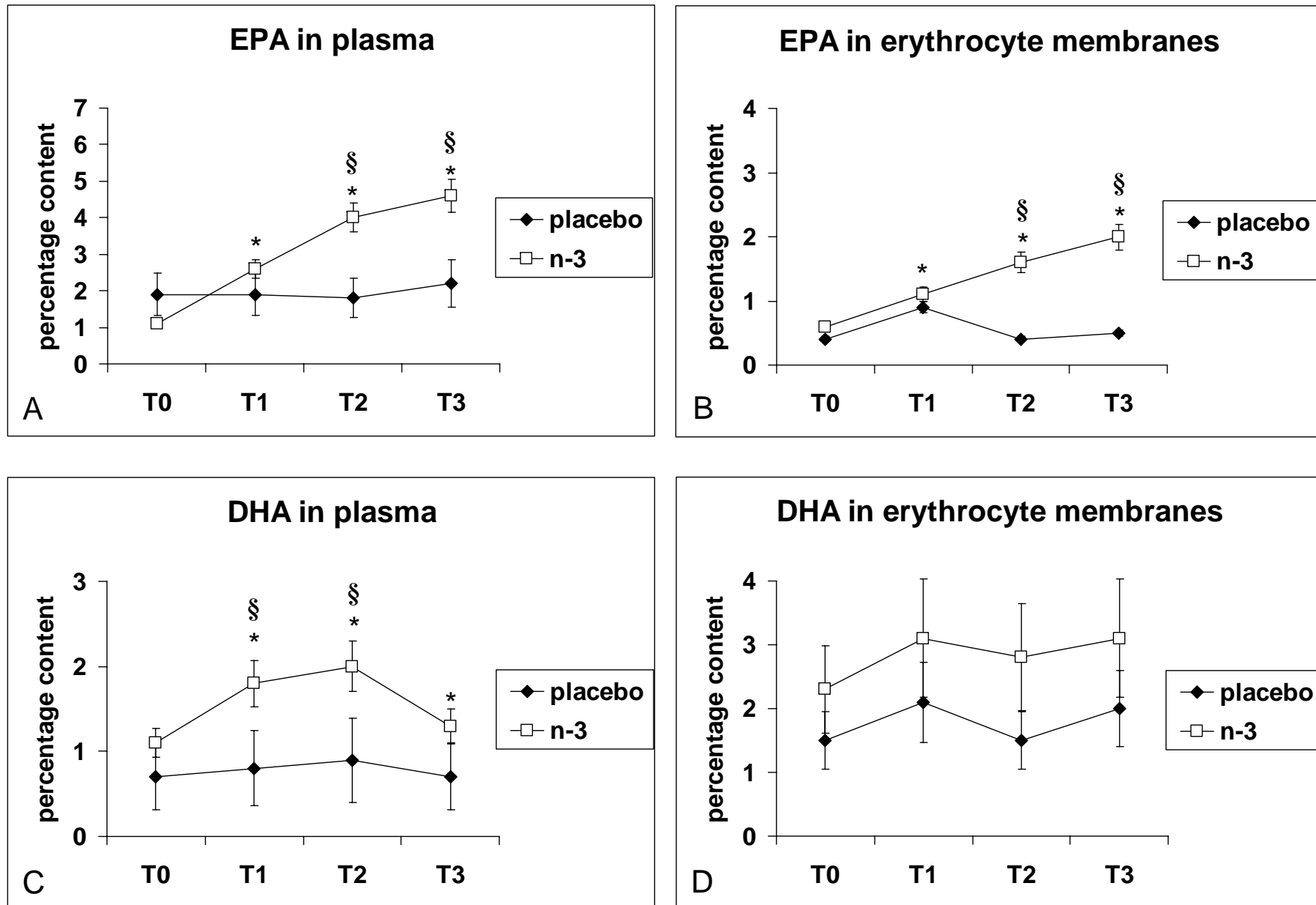


Figure 4

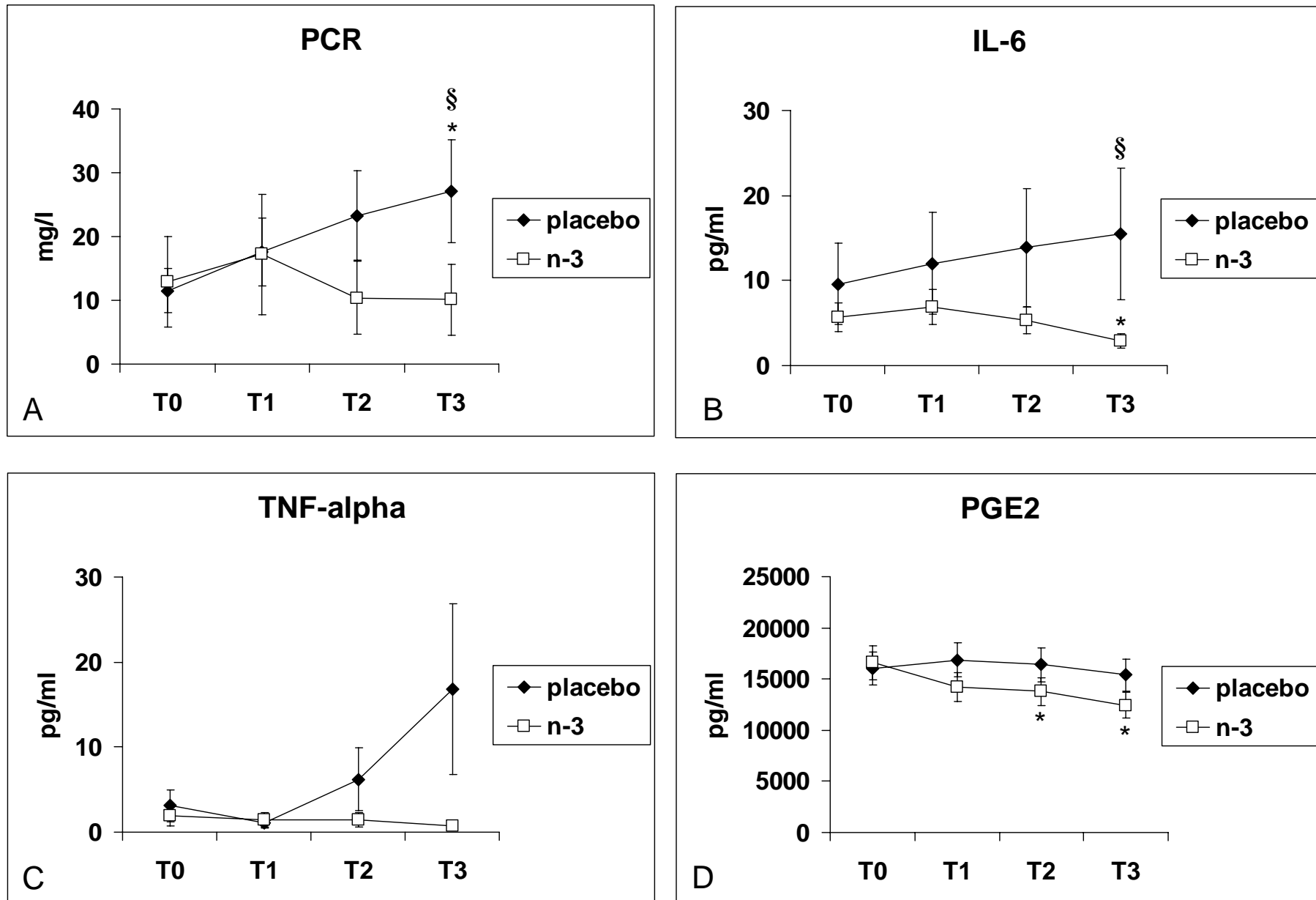


Figure 5

